

THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OF PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. A method to regulate expression of a nucleic acid sequence of interest comprising:

5 i) providing a eukaryote having:

10 1) a first nucleotide sequence comprising,

a) said nucleic acid sequence of interest operatively linked to a first regulatory region,

15 b) an operator sequence capable of binding a fusion protein, and;

2) a second nucleotide sequence comprising a second regulatory region in operative association with a nucleotide sequence encoding said fusion protein, said fusion protein comprising,

a) a DNA binding protein, or a portion thereof, capable of binding said operator sequence, and;

15 b) a recruitment factor protein, or a portion thereof, capable of binding a chromatin remodelling protein; and

ii) growing said eukaryote, wherein expression of said second nucleotide sequence produces said fusion protein that regulates expression of said nucleic acid sequence of interest.

20 2. The method of claim 1, wherein the eukaryote is a plant.

25 3. The method of claim 1, wherein in said step of introducing (step i)), said operator sequence is selected from the group consisting of a ROS operator, a Tet operator, Sin3, VP16, GAL4, Lex A, UMe6, ERF, SEBF, CBF and a DNA binding domain of a transcription factor.

30 The method of claim 1, wherein the recruitment factor is characterized as having a histone deacetylase binding domain or a histone acetylase binding domain.

4. The method of claim 1, wherein in said step of introducing (step ii)), said recruitment factor protein is selected from the group consisting of histone acetylase

recruitment factor, histone deacetylase recruitment factor, KID, ADA, SAGA, STAGA, PCAF, TFIID, TFIIIC, bnKCP1 and BnSCL1.

5. A method of enhancing expression of a nucleic acid sequence of interest comprising:

i) providing a plant with one or more constructs comprising:

1) a first nucleotide sequence comprising,

a) said nucleic acid sequence of interest operatively linked to a first regulatory region, and;

10 b) an operator sequence capable of binding a fusion protein;

2) a second nucleotide sequence comprising a second regulatory region in operative association with a nucleotide sequence encoding said fusion protein comprising,

a) a DNA binding protein, or a portion thereof capable of binding said 15 operator sequence, and;

b) a recruitment factor, or portion thereof, that binds a histone acetyltransferase (HAT) protein;

ii) growing said plant, and

iii) expressing said second nucleotide sequence such that said fusion protein is 20 produced and expression of said nucleic acid sequence of interest is increased.

6. The method of claim 5, wherein the second regulatory region comprises an inducible promoter.

25 7. The method of claim 5, wherein the HAT is Gcn5.

8. The method of claim 5, wherein in said step of introducing (step i)), said operator sequence is selected from the group consisting of a ROS operator, a Tet operator, Sin3, VP16, GAL4, Lex A, UMe6, ERF, SEBF, CBF and a DNA binding 30 domain of a transcription factor.

9. A method for selectively controlling the transcription of a nucleic acid sequence of interest, comprising:

- i) providing a first plant comprising a first genetic construct, said first genetic construct comprising a first regulatory region operatively linked to a nucleic acid sequence of interest and at least one ROS operator sequence capable of controlling the activity of said first regulatory region;
 - 5 ii) providing a second plant comprising a second genetic construct, said second genetic construct comprising a second regulatory region in operative association with a nucleic acid molecule encoding a fusion protein comprising a ROS repressor, or a fragment thereof, and a recruitment factor characterized as having a histone deacetylase binding domain, or a fragment thereof;
 - 10 iii) crossing said first plant and said second plant to obtain progeny, said progeny comprising both said first genetic construct and said second genetic construct, and characterized in that the expression of said second genetic construct represses expression of said first genetic construct.
- 15 10. The method of claim 6, wherein said first and second regulatory regions are either the same or different and are selected from the group consisting of a constitutive promoter, an inducible promoter, a tissue specific promoter, and a developmental promoter.
- 20 11. The method of claim 1, wherein, in said step of introducing (step i)), said first, second, or both said first and second nucleotide sequences are incorporated into said plant by crossing.
- 25 12. The method of claim 8, wherein said crossing comprises crossing a first plant comprising said first nucleotide sequence with a second plant comprising said second nucleotide sequence, to obtain progeny.
- 30 13. The method of claim 1, wherein, in said step of introducing (step i)), said first, second, or both said first and second nucleotide sequences are incorporated into said plant by transformation.
14. A method to regulate expression of an endogenous nucleic acid sequence of interest comprising:

i) providing a eukaryote having a nucleotide sequence comprising, a regulatory region, operatively linked with a nucleotide sequence encoding a fusion protein, said fusion protein comprising,

- 5 a) a DNA binding protein, or a portion thereof, capable of binding a segment of a DNA sequence of said endogenous nucleotide sequence of interest; and
b) a recruitment factor protein, or a portion thereof; and

ii) growing said eukaryote, wherein expression of said nucleotide sequence produces said fusion protein that regulates expression of said endogenous nucleic acid sequence of interest.

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15. The method of claim 11, wherein in said step of introducing (step i)), said recruitment factor protein is selected from the group consisting of histone acetylase recruitment factor, and histone deacetylase recruitment factor.

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16. An isolated nucleic acid sequence encoding the sequence of bnKCP1 (SEQ ID NO:71).

17. An isolated nucleic acid sequence encoding amino acids 1 to 80 of SEQ ID NO:71.

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18. An isolated nucleic acid sequence encoding amino acids 1 to 160 of SEQ ID NO:71.

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19. An isolated nucleic acid sequence encoding amino acids 81 to 215 of SEQ ID NO:71.

20. The method of claim 1, wherein the recruitment factor protein is bnKCP1 (SEQ ID NO:71) or a fragment thereof.

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21. The method of claim 11, wherein the recruitment factor protein is bnKCP1 (SEQ ID NO:71) or a fragment thereof.

22. An isolated nucleic acid encoding a bnKCP1 fusion protein, GAL4DB-bnKCP1.

23. An isolated nucleic acid encoding a HDAC fusion protein, GAL4DB-HDAC.
24. An isolated nucleic acid sequence encoding the sequence of BnSCL1 (SEQ ID NO:81).
- 5 25. An isolated nucleic acid sequence encoding amino acids 1 to 358 of SEQ ID NO:81.
- 10 26. An isolated nucleic acid sequence encoding amino acids 1 to 261 of SEQ ID NO:81.
27. An isolated nucleic acid sequence encoding amino acids 1 to 217 of SEQ ID NO:81.
- 15 28. An isolated nucleic acid sequence encoding amino acids 146 to 358 of SEQ ID NO:81.
29. The method of claim 1, wherein the recruitment factor protein is BnSCL1
- 20 (SEQ ID NO:81) or a fragment thereof.
30. The method of claim 11, wherein the recruitment factor protein is BnSCL1 (SEQ ID NO:81) or a fragment thereof.
- 25 31. A method to regulate expression of a nucleic acid sequence of interest in a plant comprising:
- i) introducing into said plant:
- 30 1) a first nucleotide sequence comprising,
- a) said nucleic acid sequence of interest operatively linked to a first regulatory region,
- b) an operator sequence capable of binding a bnKCP-fusion protein, and;

2) a second nucleotide sequence comprising a second regulatory region in operative association with a nucleotide sequence encoding said bnKCP-fusion protein, said bnKCP-fusion protein comprising,

- 5 a) a DNA binding protein, or a portion thereof, capable of binding said operator sequence, and;
- b) a bnKCP1, or a portion thereof; and

ii) growing said plant, wherein expression of said second nucleotide sequence produces said fusion protein that regulates expression of said nucleic acid sequence of interest.

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32. A method to regulate expression of a nucleic acid sequence of interest in a plant comprising:

i) introducing into said plant:

- 15 1) a first nucleotide sequence comprising,
- a) said nucleic acid sequence of interest operatively linked to a first regulatory region,
- b) an operator sequence capable of binding a BnSCL-fusion protein, and;

20 2) a second nucleotide sequence comprising a second regulatory region in operative association with a nucleotide sequence encoding said BnSCL-fusion protein, said BnSCL-fusion protein comprising,

- 25 a) a DNA binding protein, or a portion thereof, capable of binding said operator sequence, and;
- b) a BnSCL1, or a portion thereof; and

ii) growing said plant, wherein expression of said second nucleotide sequence produces said fusion protein that regulates expression of said nucleic acid sequence of interest.

33. A method of increasing cold tolerance in a plant, comprising:

- 30 i) providing a plant having a nucleotide sequence of interest operatively linked to a first regulatory region, the nucleotide sequence of interest encoding bnKCP1, or fragments thereof; and
- ii) maintaining the plant under conditions where bnKCP1 is expressed thereby increasing cold tolerance in the plant.

34. A method of controlling expression of a nucleic acid sequence of interest, comprising:

i) providing a eukaryote having:

- 5 1) a first nucleotide sequence comprising
- a) said nucleic acid sequence of interest operatively linked to a first regulatory region,
 - b) an operator sequence capable of binding a fusion protein, and
 - c) a second regulatory region in operative association with a nucleotide sequence encoding said fusion protein, the fusion protein including a DNA binding protein, or a portion thereof, capable of binding said operator sequence and a recruitment factor protein, or a portion thereof, capable of binding a chromatin remodelling protein; and
- 10 2) a second nucleotide sequence comprising a third regulatory region in operative association with a nucleotide sequence encoding a chromatin remodelling protein; and
- 15 ii) growing said eukaryote, wherein expression of said first nucleotide sequence produces said fusion protein that increases expression of said nucleic acid sequence of interest and wherein expression of said second nucleotide sequence produces said chromatin remodelling protein to repress expression of said nucleic acid sequence of interest.

20 35. The method of claim 34, wherein the chromatin remodelling protein is HDA19.

25 36. The method of claim 35, wherein the recruitment factor protein is BnSCL1 or bnKCP1.

30 37. The method of claim 35, wherein the DNA binding protein is VP16 or GAL4.

38. A method of controlling expression of a nucleic acid sequence of interest, comprising:

i) providing a eukaryote having:

- 1) a first nucleotide sequence comprising,

- a) said nucleic acid sequence of interest operatively linked to a first regulatory region, and
 - b) an operator sequence capable of binding a fusion protein, and
- 2) a second nucleotide sequence comprising a regulatory region in operative association with a nucleotide sequence encoding said fusion protein, the fusion protein including a DNA binding protein, or a portion thereof, capable of binding said operator sequence and a recruitment factor protein, or a portion thereof, capable of binding a chromatin remodelling protein; and
- 10 2) a third nucleotide sequence comprising a third regulatory region in operative association with a nucleotide sequence encoding a chromatin remodelling protein; and
- ii) growing said eukaryote, wherein expression of said second nucleotide sequence produces said fusion protein that increases expression of said nucleic acid sequence of interest and wherein expression of said third nucleotide sequence produces said chromatin remodelling protein to repress expression of said nucleic acid sequence of interest.
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39. The method of claim 38, wherein the chromatin remodelling protein is HDA19.
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40. The method of claim 39, wherein the recruitment factor protein is BnSCL1 or bnKCP1.
- 25 41. The method of claim 39, wherein the DNA binding protein is VP16 or GAL4.